The Light Microscopic Study of Age-Associated Changes in Dog Synovial Membrane Cells

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Abstract: Age associated structural changes in the synovial membrane cells were studied in 24 Kangal hybrid-breed dogs. Dogs were grouped in 3 age groups young, middle aged and old. Synovial intima were made of 1-2 layered flat and oval intimal cells in the young group dogs and 3-5 to 2-6 multi-layered cubic and polygonal lining cells in the middle aged and the old dogs. Continuous villi were not present in all the age groups and therefore, it was not possible to determine any change in the amounts of villi in different age groups. Subintima changed from areolar, adipose, areola-adipose and fibrous connective cells, from young to old group dogs. Fibroblasts and various connective tissue cells of the young dogs placed in the deep layers of the subintima. Lymphocytes and plasma cells occurred in the environs of blood vessels. Various connective tissue cells concentrated among the collagen fibers and collagen bundles in the vicinity of blood vessels in middle aged group dogs. In the old group dogs, connective tissue cells decreased in subintima and placed among collagen and elastic fibers.

Key words: Dog synovial membrane, age-associated changes, intimal lining cells, fibroblasts, mast cells, lymphocytes, plasma cells

INTRODUCTION

Different aspects of synovial membrane in human and other animals have been studied extensively. The morphology and functions of synovial membrane cells were studied by Castor (1960), Fawcett (1986), Ghardiali and Roy (1966), Krey et al. (1973), Levick and McDonald (1989), Rittig et al. (1992), Roy and Ghadiali (1967), Shizumizu et al. (1996), Shively and van Sickle (1977), Watanabe et al. (1974) and Wyllie et al. (1964). The pathology of the membrane during rheumatic illnesses were investigated by Bleedorn et al. (2011), Chew et al. (1950), Hayashi et al. (2004), Henderson and Pettipher (1985), Knight and Levick (1984), Levick and McDonald (1989), Malone et al. (1991), Rittig et al. (1992), Roy et al. (1966), Schumacher (1969), Thompson and Stockwell (1983), Watanabe et al. (1974) and Wright et al. (1989).

Morphological structure of a normal synovial membrane (synovium) is made of synovial intima (intima) subsynovial tissue (subintima, subsynovium) and stromal layers. However, synovial membrane shows variations in different mammalian animals (Bleedorn et al., 2011; Cabrera et al., 2008, Canpolat and Sagiroglu, 2000; Castor, 1960; Comerford et al., 2006; Fawcett, 1986; Hayashi et al., 2004; Henderson and Pettipher, 1985; Krey et al., 1973; Levick and McDonald, 1989; Schumacher, 1969; Shizumizu et al., 1996; Shively and van Sickle, 1977; Watanabe et al., 1974; Wright et al., 1989; Wyllie et al., 1964, Wynne-Roberts and Anderson, 1978).

In addition, many changes and abnormalities associated with aging and several disease occur in morphology and structure of synovial membrane (Bleedorn et al., 2011; Canpolat and Sagiroglu, 2000; Castor, 1960; Chew et al., 1990; Fawcett, 1986, Henderson and Pettipher, 1985; Knight and Levick, 1984; Levick and McDonald, 1989; Malone et al., 1991; Rittig et al., 1992; Roy et al., 1966; Schumacher, 1969; Shively and van Sickle, 1977; Thompson and Stockwell, 1983; Watanabe et al., 1974; Wright et al., 1989; Wyllie et al., 1964, Wynne-Roberts and Anderson, 1978).

Recent studies on synovial membrane have concentrated on electron microscopic investigations of ultrastructure, changes caused by illnesses, ruptures and treatment (Bleedorn et al., 2011; Cabrera et al., 2008; Castor, 1960; Comerford et al., 2006; Hayashi et al., 2004; Rittig et al., 1992; Roy et al., 1966; Shizumizu et al., 1996; Wright et al., 1989; Wynne-Roberts and Anderson, 1978). Therefore, studies on age associated changes in synovial membrane have prime importance in understanding of rheumatic and other age related illnesses in both human and animals (Bleedorn et al., 2011; Canpolat and Sagiroglu, 2000; Castor, 1960; Fawcett, 1986; Hayashi et al., 2004; Henderson and Pettipher, 1985; Iliani and Ghardiali, 1986; Rittig et al., 1992; Shively and
van Sickle, 1977; Watanabe et al., 1974; Wright et al., 1989; Wyllie et al., 1964; Wynne-Roberts and Anderson, 1978). In this study, age associated changes in the synovial membrane of dogs were investigated and described.

MATERIALS AND METHODS

Twenty four Kangal hybrid dogs, 12 male and 12 female were used in this experimental work. They were grouped in three age groups:

- Young animals were 0-3 months old and their weights varied between 2700-3500 g. Five dogs were in this group.
- Middle aged animals were 3.5-6 months old and their weights were between 7-14 kg (6 dogs).
- Old and very old animals were 7 months to 6 years old and their weights 10-33 kg (12 dogs).

Grouping of the dogs was made with the aid of veterinarian and earlier research on similar subjects (Campolat and Sagiroglu, 2000; Jilani and Ghadially, 1986; Thompson and Stockwell, 1983). The dogs were killed by intra-cardiac injection of nembutal. Then, left and right knee joints of the dogs were incised. Supra-patellar tendons, quadriceps and other muscle connections and joint capsule were incised. The flaps containing the patella were reflected and synovial membranes over the medial and lateral of the infra-patellar pad and parapatellar region were collected rapidly (Campolat and Sagiroglu, 2000; Castor, 1960; Chew et al., 1960; Ghadially and Roy, 1966; Jilani and Ghadially, 1986; Knight and Levick, 1984; Krey et al., 1973; Levick and McDonald, 1989; Roy et al., 1966; Roy and Ghadially, 1967; Schumacher, 1969; Thompson and Stockwell, 1983; Watanabe et al., 1974; Wyllie et al., 1964).

Tissues were placed on filter paper as intimal layers positioned on top (Campolat and Sagiroglu, 2000; Ghadially and Roy, 1966; Jilani and Ghadially, 1986; Krey et al., 1971, 1973; Levick and McDonald, 1989; Roy et al., 1966; Roy and Ghadially, 1967).

For microscopy, tissues were fixed in 10% formalin and Helly’s solution, dehydrated in increasing concentrations of ethanol, cleared in xylol and embedded in paraffin (Jilani and Ghadially, 1986; Luna, 1968; Roy et al., 1966). Total 5-6 μm thick synovial membrane sections were prepared and stained. The stains used were Haematoxylin-Eosin, Crossmon’s triple stain, Dominici’s stain, PAS, Resorsin-Fuchs in, Klaver-Barrera and Ironed HE. Stained sections were studied using BH 2-Olympus photomicroscope (Bradbury and Gordon, 1977; Crossmon, 1937; Dubrey and Rack, 1970; Luna, 1968, McMamurs and Mowry, 1960).

RESULTS AND DISCUSSION

First group (young dogs, 0-3 months old): Synovial membrane appeared white, smooth, thin and glistening, macroscopically. Intimal layer of the articular cavity formed of 1-2 layers of intimal cells (Fig. 1). These cells were platy or oval shaped fibroblast-like and macrophage-like cells (Fig. 1 and 2). Basal membrane was absent under the cells (Fig. 2). Membrane villi exhibited various shapes and extended into articular cavity as 1-3 layered or 2-5 layered intimal cells (Fig. 3).

Although, subsynovial (subintimal) layers of this group of dogs showed changes from the surface towards depths, areolar were adipose, fibrous and dominantly areola-adipose types (Fig. 1-3). Fibroblasts and various connective tissue cells placed in deeper layers of subintima and lymphocytes and plasma cells were close to blood vessels (Fig. 1-3).

Fig. 1: Synovial membrane from young (0-3 months old), dogs. Synovial Membrane (SM) Intima (In) towards articular cavity is made of 1-2 layered and platy or round shaped cells. Any basement membrane under the cell layer is absent. Intimal cell layer extends towards the articular cavity as Areola-Adipose (Ar-Ad) connective tissue bearing Villus (Vil). In the top shallow layer (I) of Subintima (Sin), Collagen fibres (Co) are present as scattered or clustered. The deeper layers (II) include Areolar (Ar) and Adipose (Ad) connective tissues. In the deepest layers (III), Collagen fibres form bundles (CoB) and Fibroblasts (F) with various connective tissue cells are present. Haematoxylin-Eosin, x40
Fig. 2: Appearance of the synovial membrane of dogs of the first group with a greater magnification. Fibroblast-like (F-I) and Macrophage-like (M-I) cells are openly visible in Intima (In). Blood vessels of different diameters such as Capillary (Ca) and Arteriole (A) are seen in superficial subintima layer (I) just under intimal cells. This layer (I) include collagen fibres as either scattered (Co) or small bundles (CoB). Around the blood vessels; Lymphocyte (L) and Plasma cells (P) are also present. The deeper subintima (II) in areolar and adipose subintima character and rarely bears cells [e.g., Fibroblast (F)] and Capillaries (Ca). Crossman’s triple, x100

In young dog synovial membranes, granulated mast cells were observed as placed close to fibrous and areola adipose connective tissues and were rare in the adipose connective tissues (Fig. 4).

Second group (middle aged animals 3.5-6 months old): This group of dogs had 1-2 or 3-5 layered intimal lining cells. Nuclueses of the intimal cells were rather flattened (Fig. 5 and 6). The membrane villi exhibited various shapes and sizes (Fig. 5). In the structure of villi and villus absent synovium samples subintima contained areolar, adipose and areola-adipose connective tissues (Fig. 5-7).

Various connective tissue cells occurred as placed between collagen fibres and the bundles and peripherally accumulated around the vascular structures (Fig. 5-7). A large cell, probably a mast cell was seen as placed on arteriole wall (Fig. 7).

Third group (Old group 7 months to 6 years old animals): The synovial membranes of this group had a pastel greyish colour and rough surface, macroscopically. The distinctive feature observed in microscopic studies was the shapes of the cells although platy cells were present in places cell shapes were dominantly cubic and polygonal (Fig. 8 and 9). They were probably Fibroblast-like (F-I) and Macrophage-like (M-I) cells and especially in villi less layered. The intimal layer, on the contrary, formed of 2-6 layers of cells (Fig. 8 and 9). In synovial membranes of old animals group, the number of villi were much higher than those of young group and the villi were various shaped and had extensions (Fig. 9). Synovial intima of villi occurred as lined either with dense and multi-layered cells or scarce cells, in accordance with sections directions. The intimal cells did not have a basal membrane (Fig. 9).

In comparison to the first and the second group animals, the subintima of old animals group were areolar and more fibrous connective tissue character (Fig. 8 and 10).

Minor amounts of connective tissue cells scattered among collagen and elastic fibres were observed.
especially in fibrous subintima (Fig. 8 and 10). In the deeper parts of old synovial membrane subintima, mast cells were observed collective groups (Fig. 11).

Studies on human synovial membrane have been unable to confirm the existence of age associated distinct changes (Castor, 1960; Rittig et al., 1992; Roy et al., 1966; Wright et al., 1989; Wynne-Roberts and Anderson, 1978).

The most studies on animal and human synovial membranes were on young subjects and therefore age associated changes could not be investigated (Bleedorn et al., 2011; Cabrera et al., 2008; Castor, 1960; Comerford et al., 2006; Fawett, 1986; Ghadially and Roy, 1966; Hayashi et al., 2004; Henderson and Pettipher, 1985; Krey et al., 1971, 1973; Levick and McDonald, 1989; Roy and Ghadially, 1967; Schumacher, 1969; Shimizu et al., 1996; Shively and van Sickle, 1977; Thompson and Stockwell, 1983; Watanabe et al., 1974; Wright et al., 1989; Wyllie et al., 1964; Wynne-Roberts and Anderson, 1978).

Light microscopic studies on age associated changes in the synovial membrane are very scarce (Canpolat and Sagiroglu, 2000; Castor, 1960; Jilani and Ghadially, 1986; Levick and McDonald, 1989; Rittig et al., 1992; Wynne-Roberts and Anderson, 1978). The outstanding researches of Jilani and Ghadially (1986). All of these researches are on age associated changes in rabbit synovial membrane.

This study investigates dog synovial membrane with light microscopic methods as a whole. Recent researches on dog synovial membrane are generally on the changes related disease, ruptures and medical treatments (Bleedorn et al., 2011; Cabrera et al., 2008; Canpolat and Sagiroglu, 2000; Castor, 1960; Comerford et al., 2006; Hayashi et al., 2004; Henderson and Pettipher, 1985). Therefore, this study will contribute a good deal in understanding age associated changes in the synovial membrane of dog.

**Macroscopic appearance:** The synovium of the young animals were pearly white coloured, smooth, glistening
Fig. 6: Microscopic appearance of second group (middle aged) dogs’ synovial membrane. Synovial Intima (In) was formed of 1-2 layered cells and cell nucleuses are flattened. Collagen fibres (Co) just under intima were rather concentrated. Collagen fibre Bundles (CoB) were seen in the Areolar connective tissues (Ar) of deeper layers (I and II) and, in the Areola-Adipose (Ar-Ad) and Adipose (Ad) of subintima. An artery (Ok) in the deep subintima had Internal elastic Membrane (IeM) and surrounding it a Venule (Ve) and Arterioles (A) were present. Collagen fibre Bundles (CoB) and various connective tissues were seen as concentrated around blood vessels. Crossman’s triple, x20

Fig. 7: Deeper subintima appearance of middle aged group dogs. Subintima was lined with Areolar (Ar) and in places Adipose (Ad) connective tissues. In comparison with young group dogs, vascularity increased in the deeper parts of subintima. A large, probably a mast, cell (pointed with double arrow) was present close to Arteriole (A) wall. Haematoxylin-Eosin, x20

Fig. 8: Microscopic view of synovial membrane of the old aged (7 months to 6 years old) group dogs. The Intimal cell layer (In) was formed of 3-6 layered cubic and polygonal cells. Among the multi-layered intimal cells Capillaries (Ca) were present. The Subintima (SIn) was transformed to Fibrous connective tissue (Fi). In subintimal layer, Capillaries (Ca) among various connective tissue cells and blood vessels (Ok) with increasing radii with depth were present. Collagen fibres (Co) and Collagen fibre Bundles (CoB) were concentrated. Haematoxylin-Eosin, x40

the synovium were greyish coloured, rough and pastel. Similar descriptions made by Jilani and Ghadially (1986).

**Intima:** Synovial intimal layer generally observed as made of 1-2 layered cells in young dogs. Intimal cells (fibroblast-like and macrophage-like) flattened or oval shaped. In middle aged group, intima contained 1-2 layered or 3-5 layered lining cells. Synovial intima of the old group formed of 2-6 layered cells. Intimal cell shapes of second and third groups were cubic or polygonal. However, some studies claimed that the thicknesses of synovial cell layers were not associated with the age (Castor, 1960). Synovial intima of juvenile human is 1-3 layered, chubby and formed of rounded and oval shaped cells. The cells were classified in three Groups A-C cells (Wynne-Roberts and Anderson, 1978).

The synovial membranes of adult rabbits are made of 1-3 layered lining cells. The membrane cells are described as A (macrophage-like, M), B (fibroblast-like, F) and C (intermediate, I) type cells and discriminating of these cells in rabbits is easier than in human (Krey et al., 1973).
Fig. 9: Microscopic view of Synovial Membrane (SM) of old group dogs. Section was through crowded and variously shaped Villus (Vil). Intima (In) contained flattened cells in places. The dominant cell shapes were cubic and polygonal shaped. PAS stained sections showed that intimal cells (F-I and M-I) did not have basement membrane. The villus had both cell free and cell crowded parts. Collagen fibres (Co) were scattered and placed among intima. The diameters and numbers of Capillaries (Ca) and other blood vessels (arrow) increased gradually towards the deeper parts of subintima (Sin). PAS, x40. Periodic Acid Schiff reaction, x40.

Jilani and Ghadially (1986) concluded that B and AB (C) type synovial cells are present in animals and with aging of rabbits A cells increase and B cells decrease in evident amounts. Traumatic effusions in the synovial membrane cause the disappearance of the differences between A and B type cells (Roy et al., 1966). The synovial intima is separated from the subintimal tissue by an intermediate fibrillar zone rich in staining for type III collagen in old human donors (69-94 years of age) (Kittig et al., 1992).

Villi: Synovial villus appearances differed in various sections. Continuous villi were absent and any noticeable increase or decrease in number of villi in different age groups was not detected. However, normal synovial tissue villi were numerous in all three age groups. The villi showed variations both in shapes and number of layers according to locations of sections. In young animals, villi were extending as bung bodies towards articular cavity. The intimal lining cells were 1-3 or 2-5 layered. In general, their inner parts were lined with areolar connective tissues. The villi of middle and old group dogs were in various shapes and sizes. In old dogs, villi were plenty and lined with multi-layered cells. Jilani and Ghadially (1986), claimed that villi were not noticeable in normal synovial membrane of human and they increased with aging and disease. But they could not observe the villus in rabbits. This is due to the difference of species. In this study, synovial filopodia were not detected. Filopodias

Fig. 10: Appearance of deeper parts of synovial membrane of old group dogs. Subintima changed to Areolar (Ar) and Fibrous (Fi) connective tissues. Collagen fibres were as thick bundles (CoB) and bands (arrow heads). Elastic fibres (El) increased and thickened in both general structure of synovial membrane and in the walls of blood vessels. Connective tissue cells were present between a large blood vessel (arrow) and collagen and elastic fibres. Resonsin-Fuchsin, x20.

Fig. 11: Appearance of the mast cells placed deep in the Subintima layer (Sin) of the Synovial Membrane (SM) of the old group dogs. The mast cells (double arrows) occurred as aggregated and some degranulated. Ironed HE, x100.
were detected in several studies on the subject (Ghadially and Roy, 1966; Roy and Ghadially, 1967; Watanabe et al., 1974; Wyllie et al., 1964).

**Basement membrane and tight junctions:** Intimal lining cells did not have a basement membrane in vilius and other synovial parts of all three groups of the dogs. Henderson and Pettipher (1985) did not observe basement membrane and desmosomes in human synovium. In addition, Knight and Levick (1984), Thompson and Stockwell (1983) and Watanabe et al. (1974) did not observe tight junctions and desmosome-like structures in rabbit synovial lining cells.

In other studies (Roy and Ghadially, 1967; Wyllie et al., 1964; Wynne-Roberts and Anderson, 1978), desmosome-like structures were described in synovial membranes of human and experimental animals.

In the intima basement, membrane-like material associated with synovial lining cells stains for type IV collagen and laminin in old human donors (69-94 years of age) (Rittig et al., 1992).

**Subintima:** Despite some differences due to the locations of the sections taken, from the top towards depths of subintima of the young dogs, the connective tissues were as in areolar, adipose and areola-adipose types. In comparison with synovial membranes of young and middle aged groups, in the synovial membranes of the old group subintima areolar usually changed to fibrous connective tissues. Juvenile human synovium is in areolar and fibro-areolar types and is similar to adult human and animal synovium (Wynne-Roberts and Anderson, 1978).

In experimental animals synovial membrane shows many structural variations and these variations evolve into complex formations with aging (Fawcett, 1986; Jilani and Ghadially, 1986; Shivley and van Sickle, 1977). It was noted that subintima type could change depending on the functions of and pressures and impacts on the synovium (Fawcett, 1986; Henderson and Pettipher, 1985).

**Connective tissue cells/mitotic figures:** Fibroblasts and various connective tissue cells of studied young group dogs occurred as placed primarily in deeper layers of subintima. The lymphocyte and plasma cells were in the neighbourhood of blood vessels, in the young group. In the middle aged group, various connective tissue cells were seen as collagen fibres and bundles around blood vessels. In the fibrous subintima of the old group, the connective tissue cells decreased and the cells emplaced among collagen and elastic fibres.

Juvenile and adult human synovium were described as similar by Wynne-Roberts and Anderson (1978). In animals, the connective tissue cells were described in different structures and various changes with aging (Campolati and Sagiroglu, 2000; Krey et al., 1971; Thompson and Stockwell, 1983).

The mitotic figures were not detected in normal synovial tissue (Castor, 1960). However, Krey et al. (1971) and Thompson and Stockwell (1983) determined some mitotic figures.

**Mast cells:** In young the group dogs few degranulated type mast cells were present among intimal and subintimal cells of the synovial membrane. In this group, these cells were common in fibrous and areola-adipose connective tissues and were rare in adipose tissues.

In the middle aged animals, the mast cells were placed close to blood vessel walls. In old animals any mast cells were absent in intimal layer. In deeper layers of subintima, common aggregate of the mast cells were observed. Some of the mast cells were in degranulated type. Studies on human synovium determined that the mast cells were the most prominent (about 3% of the total) cells of the subintima. The studies found that mast cells decreased with aging and they were rare in fibrous type synovium. The mast cells placed, usually just under the intimal cell layer and as attached capillaries and fat cells (Castor, 1960).

Macrophage and mast cells were not observed in young rabbits and adult rabbit synovium contained sparse mast cells (Krey et al., 1973; Thompson and Stockwell, 1983). Normal mast cells of monkeys were quite similar to those humans in structural and dispersion manners and they placed close to blood vessels (Schumacher, 1969).

Castor (1960) in his study of variously (14-68) aged human synovial tissues concluded that although some changes were present, the changes not clearly associated with age gender and intra-articular localization.

**CONCLUSION**

A few granulated mast cells were observed between intimal and subintimal cells of the young group dogs. In middle aged group dogs, mast cells were close to blood vessel wall. The mast cells grouped in the deeper layers of subintima and some were observed as granulated, in the old group dogs.

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